VPI94-04

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Sheela J. Huff
Group	:	1811
Applicants	:	Guy W. Bemis, et al.
Serial No.	:	08/261,452
Filed	:	June 17, 1994
For	:	INHIBITORS OF INTERLEUKIN-1ß CONVERTING ENZYME

Honorable Assistant Commissioner
for Patents and Trademarks
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132 OF MARK A. MURCKO

I, MARK A. MURCKO, hereby declare and state as follows:

1. I am one of the co-inventors of the subject matter of the above-identified application.

2. I received a B.A. in Chemistry from Fairfield University in Fairfield, Connecticut in 1982. In 1987, I received a Ph.D. in Physical Organic Chemistry from Yale University, New Haven, Connecticut. After receiving my Ph.D., I worked for three years (1987 to 1990) in the Molecular Systems Group at Merck, Sharpe and Dohme Research Labs in West Point, Pennsylvania. In 1990, I began my

present research in the Molecular Modeling Group at Vertex Pharmaceuticals, Inc., Cambridge, Massachusetts ("Vertex").

I have published 30 scientific papers in peer-reviewed journals. A copy of my curriculum vitae is attached as **Exhibit A.**

3. I have devoted a major portion of my scientific work toward the study of molecular interactions such as those involved in receptor-ligand binding and enzyme-substrate recognition. In particular, I have studied molecular interactions using computer-assisted molecular modeling approaches. Publications pertaining to the above listed areas of research may be found in my curriculum vitae (**Exhibit A**).

4. Since joining Vertex in 1990, my work has been devoted to computer-assisted molecular drug design. In particular, I have modeled a variety of pharmaceutical target molecules in three-dimensional space using computer molecular modeling programs, and have then studied spatial and energetic constraints between those targets and potential interacting molecules. Since 1990, I have worked at developing three-dimensional "pharmacophore" models of enzyme active sites, and designing and selecting compounds

that can interact favorably with those sites.* In particular, I have worked on modeling the interleukin-1ß converting enzyme (ICE) pharmacophore based on ICE crystal coordinates, and in designing and selecting molecules that can inhibit ICE activity by virtue of their ability to interact favorably with several moieties that form the ICE active site.

5. I have read the September 20, 1995 Office Action in the above-identified application and the documents cited therein. I also attended a March 1, 1996 interview with Examiner Huff. I make this declaration for the following purposes:

a) to demonstrate that compounds that are ICE inhibitors <u>in vitro</u> can decrease IL-1ß steady-state levels <u>in vivo</u> and can ameliorate inflammation in a mouse with Type II collagen-induced arthritis, an accepted animal model for rheumatoid arthritis;

b) to explain the properties of the ICE pharmacophore, to compare the differences between using the

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* A pharmacophore is a model of molecular (i.e., physical, chemical and electrical) properties complimentary to the target site on a molecule, such as a ligand binding site of a receptor or an enzyme active site. Thus, the pharmacophore is a generic structural description of compounds that bind to the target site. It can be a group of atoms, or moieties such as electron or hydrogen bond donors and acceptors, having defined distance relationships within three-dimensional space.

ICE pharmacophore of our invention to the drug discovery methods of the prior art in identifying compounds that can inhibit ICE and to explain how those differences impact on the ability of those skilled in the art to identify and to design compounds that are encompassed by the claimed ICE pharmacophore and are, thus, ICE inhibitors; and

c) to demonstrate that one of ordinary skill in the art, with the ICE pharmacophore of our invention in hand, can identify and synthesize compounds that fall within the claimed pharmacophore and thus inhibit ICE -- without undue experimentation -- by following the protocols set forth in this application.

6. Prior to our invention, it was well-established in the art that IL-1ß, a cytokine released from stimulated monocytic cells, is the major form of secreted IL-1 and is an early and primary player in activating inflammation pathways associated with immune disorders and disease. As stated in D. K. Miller et al., "The IL-1ß Converting Enzyme as a Therapeutic Target," Annals N.Y. Acad. Sci., 696, pp. 133-148 (1993) (attached as **Exhibit B**),

> "[t]he importance of IL-1 as a target for antiinflammatory therapy is shown by the efficacy of IL-1RA [an IL-1 antagonist], soluble IL-1R [IL-1-receptor] and anti IL-1 receptor monoclonal antibodies in several animal models of human disease" (p. 133).

7. As another example, T. Geiger et al., "Neutralization of interleukin-1ß activity in vivo with a monoclonal antibody alleviates collagen-induced arthritis in DBA/1 mice and prevents the associated acute-phase response," Clin. Exper. Rheumatol., 11, pp. 515-22 (1993) (attached as **Exhibit C**), stated:

> "[f]rom our results we conclude that IL-1ß is the mediator predominantly responsibile for the pathophysiological disturbances in collagen arthritis, including joint destruction and the concomitant acute phase reaction" (p. 520-21).

Thus, the art at the time of our invention clearly pointed to the utility and efficacy of blocking IL-1ß signaling in treating inflammation and associated diseases and disorders. Accordingly, a number of approaches to blocking the effects of active IL-1ß are under development. Many of these approaches are designed to intervene in the inflammatory pathway after active IL-1ß is produced. Another approach, and the one we have chosen, prevents the production of active IL-1ß. Thus, it affects the inflammatory pathway at an earlier stage than the approaches that block mature IL-1ß action.

8. At the time of our invention, it was known that IL-1ß is initially produced as an inactive, 31-33 kD

cytoplasmic precursor ("pre-IL-1ß"). The precursor is then proteolytically cleaved by Interleukin-1ß Converting Enzyme (ICE) to release the 17 kD active, mature IL-1ß polypeptide. ICE is, thus, a useful target for IL-1ß-based therapeutic strategies because of its essential role in producing active IL-1ß. In fact, ICE inhibitors are useful in therapy because they block the formation of active IL-1ß. This also differentiates ICE inhibition therapy from other treatments for inflammation, which attempt to block cytokine signaling by active IL-1ß already produced and secreted from stimulated monocytic cells.

9. It was known in the art at the time of our invention from several lines of evidence that ICE inhibition did have an effect on in vitro and in vivo IL-1ß production and associated inflammation. For example, it was known that large amounts of (tetra)peptide substrates containing the ICE amino acid cleavage recognition site could decrease mature IL-1ß release from human peripheral blood monocytes (D. K. Miller et al., supra; **Exhibit B**) ("Miller-1"). In these experiments, radioactively-labeled human peripheral blood monocytes, pretreated with an ICE peptide inhibitor ("L-709,049") or a control peptide, were stimulated with heat-killed bacteria, which promotes rapid synthesis and secretion of mature IL-1ß. Mature IL-1ß release was

inhibited in a dose-responsive fashion from cells pre-treated with the ICE inhibitor peptide but not with the control peptide (see Miller-1 Figs. 11 and 12, p. 144-45).

In addition, in B. E. Miller et al., "Inhibition of Mature IL-1ß Production in Murine Macrophages and a Murine Model of Inflammation by WIN 67694, and Inhibitor of IL-1ß Converting Enzyme," J. Immunol., 154, pp. 1331-38 (1995) ("Miller-2"; attached as **Exhibit D**), chambers were implanted subcutaneously into the backs of mice and inflammatory resonses were initiated by injecting zymosan into the chambers. Fluid was then aspirated from the chambers six hours later to measure IL-1ß levels in the presence or absence of intraperitoneal injection of a peptide ICE inhibitor ("WIN 67694"). Miller-2 showed that parenteral administration of the peptide ICE inhibitor could inhibit mature IL-1ß production in vivo (Fig. 7, p. 1336).

10. There were also growing indications in the art that ICE was involved in apoptosis (programmed cell death). For example, it had been shown that overexpression of ICE could induce apoptosis in cultured cells (Miura et al., "Induction of Apoptosis in Fibroblasts by IL-1ß-Converting Enzyme, a Mammalian Homolog of the C. elegans

Cell Death gene ced-3," Cell, 75, pp. 653-60 (1993) (attached as **Exhibit E**).

11. Further evidence for the in vivo role of ICE in IL-1ß production, inflammation and apoptosis comes from recent studies in ICE-deficient "knock-out" mice, in which the gene encoding ICE has been disrupted. My co-workers at Vertex compared IL-1ß secretion from lipopolysaccharide (LPS)-stimulated monocytes from normal and ICE-deficient mice and showed that ICE-deficient knock-out mice had vastly reduced serum levels of IL-1ß (<0.5-1.0% of normal) after LPS stimulation (Kuida et al., Science, 267, pp. 2000-2003 (1995) ("Kuida"; attached as **Exhibit F**)). In addition, Kuida showed that thymocytes isolated from ICE-deficient mice were resistant to Fas antigen-mediated apoptosis.* Kuida determined the response of normal and ICE-deficient thymocytes to three apoptotic stimuli (glucocorticoid, ionizing radiation and anti-Fas antibody) and showed that ICE-deficient thymocytes were sensitive to the first two stimuli but resistant to anti-Fas antibody-triggered apoptosis compared to normal thymocytes. Together, these

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*The Fas antigen is a thymocyte surface protein which mediates apoptosis in activated T cells. Mice with mutations in the gene that encodes Fas develop lymphadenopathy and suffer from systemic autoimmune disease (see Kuida, p. 2002).

data establish a role for ICE in Fas-mediated apoptosis in normal thymocytes.

Another group has shown that ICE-deficient knock-out mice are unable to mount severe inflammatory responses after injections of high doses of LPS. In normal mice, high doses of LPS induce massive systemic release of proinflammatory cytokines that typically cause death by endotoxic shock. ICE-deficient mice, however, are resistant to such LPS-induced endotoxic shock because they fail to produce the high levels of mature IL-1ß required to mount these deadly inflammatory responses (Li et al., "Mice Deficient in IL-1ß-Converting Enzyme Are Defective in Production of Mature IL-1ß and Resistant to Endotoxic Shock," Cell, 80, pp. 401-11 (1995) (attached as **Exhibit G**)). These data show that ICE is an effective target for controlling IL-1ß levels and inflammation in vivo.

12. My co-workers at Vertex have also shown that an ICE inhibitor that reduces IL-1ß levels in vivo can have profound effects on inflammation as demonstrated in a mouse with collagen- or LPS-induced arthritis, an accepted animal model for rheumatoid arthritis in humans (G. Ku et al., "IL-1ß Converting Enzyme Inhibition Blocks Progression of Type II Collagen-Induced Arthritis in Mice," Cytokine, in press,

1996) ("Ku"; attached as **Exhibit H**). Type II collagen-induced arthritis (CIA) involves humoral and cellular immunological responses to Type II collagen, synovial inflammation, and cartilage and bone destruction similar to the symptoms presented in human rheumatoid arthritis. The CIA mouse model is described in more detail below.

In particular, Ku demonstrated that a single dose of a tetrapeptide analog ICE inhibitor ("VE-13,045"*; see Ku, Fig. 1) inhibits LPS-stimulated IL-1ß production in vivo by 50-70%. This compound also profoundly reduced the severity of inflammation and the progression of arthritis when administered in vivo to a mouse with Type II collagen-induced arthritis (CIA).

13. Specifically, CIA was induced in mice (6-10 per group) by intradermal injections with 100 mg of chick Type II collagen (CII) on day 0, followed by a 200 mg booster injection on day 21. Controls and test ICE inhibitor compounds were then administered daily by intraperitoneal injection. Front paws (ventral surface) were examined daily after the CII booster injection and the

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* The compound VE-13,045 is an ICE inhibitor which fell within former claim 1 but which is no longer encompassed by amended claim 1 because it does not comprise a poly-substituted cyclic group having between three and seven substituents. See paragraph 16, below, for experimental results in mice with CIA using compounds that fall within the amended claims.

status of arthritic disease scored for the severity of inflammation and arthritic disease within a range of 1 to 5. Level 1- erythema (abnormal redness of the skin due to capillary congestion); Level 2- focal carpal (wrist) joint swelling; Level 3- swelling of the entire wrist; Level 4- spread of swelling to the metacarpal/metatarsal (palm) region; and Level 5- swelling affecting the metacarpo-phalangeal or metatarsalphalangeal joints.

The scores of both front paws were combined and the mean +/- SEM (standard deviation) determined for each treatment group. Thus a single mouse could attain a score ranging from about 1-2 (two relatively normal paws) to a level of about 8-10 (two paws severely degenerated from inflammation). A photograph depicting single mouse front paws at increasing arthritic index levels of between 1 to 4 according to this assay is attached as **Exhibit I.**

14. Figure 3A of Ku (attached as **Exhibit J**) shows the effect of various inflammation treatments in CII-treated mice with CIA. Mice with CII-induced inflammation receiving a negative control treatment yielded a score in the range of 5-5.5 at day 24 in this assay (-■-). Mice treated with methyl prednisolone (a steroidal anti-inflammatory agent presently used to treat human inflammation) had slightly

reduced symptoms, scoring in the range of about 3.5-4.5 at day 24 (-▲-). In contrast, mice injected with the ICE inhibitor showed a dramatic decrease in inflammation and associated tissue degeneration, scoring between 2-3 at day 24 (-●-).

15. An electron micrograph of a synovial joint from a negative control-treated (A) compared to an ICE inhibitor-treated arthritic mouse paw (B) is attached as **Exhibit K.** These photographs show that the paws of negative control-treated mice (A) with arthritic index scores of between 4 to 5 have joint synovial spaces occluded by severe inflammation as compared to paws of ICE inhibitor-treated mice (B) with arthritic index scores of between 1 to 2.

16. Recent results from mouse paw arthritis tests performed using three ICE inhibitor compounds that fall within the pending claims are shown in **Exhibit L** (A-C, attached).

Specifically, sheet (A) of **Exhibit L** shows the effects of administering by intraperitoneal injection twice daily either vehicle alone (5 ml/kg; (-■-)(the negative control)) or ICE inhibitor compound 217e (5 mg/kg; (-●-) (a compound which falls within the pending claims)) on the combined severity arthritic score of front mouse paws with

increasing time (days) post-booster with Type II collagen (CII). Mice with CII-induced inflammation receiving a negative control vehicle treatment yielded a score in the range of about 4-4.5 at day 13 in this assay (-■-). In contrast, inflamed mice injected with the ICE inhibitor 217e showed a decrease in inflammation and associated tissue degeneration, scoring between 2-2.5 at day 13 (-●-). See **Exhibit I** for visual comparisons of the arthritic symptoms.

17. Sheet (B) of **Exhibit L** shows the effects of orally administering twice daily either vehicle alone (5 ml/kg; (-■-) (the negative control)) or ICE inhibitor compound 213e (150 mg/kg; (-●-) (a compound which falls within the pending claims)) on the combined severity arthritic score of front mouse paws with increasing time (days) post-booster with Type II collagen (CII). In particular, this experiment shows that mice with CII-induced inflammation receiving a negative control vehicle treatment yielded a score in the range of about 3.5 at day 19 in this assay (-■-). In contrast, inflamed mice injected with the ICE inhibitor 213e showed a dramatic decrease in inflammation and associated tissue degeneration, scoring between 1-2 at day 19 (-●-). See **Exhibit I** for visual comparisons of the arthritic symptoms.

18. Sheet (C) of **Exhibit L** shows the effects of orally administering twice daily either vehicle alone (5 ml/kg; (-■-) (the negative control)) or ICE inhibitor compound 2100e (a compound which falls within the pending claims) at 75 mg/kg (-◆-) or 150 mg/kg (-●-) on the combined severity arthritic score of front mouse paws with increasing time (days) post-booster with Type II collagen (CII). In particular, this experiment demonstrates that the severity of arthritis symptoms in mice with CIA treated with the ICE inhibitor is reduced in a dosage-dependent manner.

19. Ku and the additional data in **Exhibit L** show that administration of an ICE inhibitor is also effective in reducing and alleviating inflammatory-associated symptoms and disease states in vivo.

20. My co-inventors and I have now tested in vitro hundreds of ICE inhibitor compounds selected using the ICE pharmacophore according to our invention (see below) that fall within the pending claims. The vast majority are active ICE inhibitors. We are now in the early stages of testing these ICE inhibitors in vivo. A summary of our collective data for several of these ICE inhibitor compounds (i.e., those which have been carried from in vitro to in vivo testing) is attached (**Exhibit M**).

21. Specifically, **Exhibit M** (col. 2) shows the in vitro inhibition constants* for eight ICE inhibitor compounds (col. 1) according to our invention. The corresponding whole cell inhibition constants** (measured in isolated whole PBMC cells and in non-isolated whole cells measured from human blood directly), are shown in columns 3 and 4, respectively. Our conclusion is that compounds that inhibit ICE enzymatic activity in vitro (col. 2) also inhibit IL-1ß production in whole cell assays (cols. 3 and 4). Furthermore, the more effective in vitro ICE inhibitors (lower inhibition constants) tend also to be better IL-1ß inhibitors in the whole blood cell assays.

22. The data displayed in **Exhibit M** also show that ICE inhibitors that have in vitro activity are able to inhibit IL-1ß production in vivo after oral administration (col. 5; "PO") or intraperitoneal injection (col. 6; "IP"). The ability of an in vitro ICE inhibitor to inhibit IL-1ß production effectively in vivo is, in part, a function of its in vivo stability or "half-life." Thus, for example,

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* The in vitro inhibition constant (K_i(nM) UV-visible) is the concentration of compound required to inhibit ICE activity by 50% as calculated from enzyme assays monitoring the cleavage of fluorescent substrate. A lower number thus represents a better inhibitor.

** The whole cell inhibition constant (IC50 (nM)) is the concentration of compound required to reduce the steady-state serum level of IL-1ß by 50% as calculated using an ELISA (enzyme-linked immunoadsorption assay).

compounds having similar in vitro inhibition constants but higher clearance rates may be less effective ICE inhibitors in vivo. Likewise, the relative abilities of two compounds to inhibit ICE in vivo may parallel their relative abilities in vitro if they have similar in vivo clearance rates. **Exhibit M** shows that this correlation holds true for compounds 214e and 265 (compare cols. 2-7). These compounds have the same clearance rate in a mouse (col. 7). Compound 214e is a better in vitro ICE inhibitor than compound 265 (col. 2), and inhibits IL-1ß production in vivo approximately 75-78% compared to 27-30% for compound 265 (cols. 5 or 6). Thus, for these compounds, as expected, lower in vitro inhibition constants correlate with greater in vivo inhibitory activity when the in vivo clearance rates are similar.

23. The data described above confirm that ICE is an important target for the design of anti-inflammatory and disease modifying drugs and that the claimed pharmacophore defines ICE inhibitors by structure. Prior to our invention, however, there was little guidance as to how to identify and design compounds which were ICE inhibitors. Peptides containing the ICE tetrapeptide cleavage recognition site of the pre-IL-1ß substrate were the usual starting points, but there was little guidance as to how to

modify the structure of this tetrapeptide to design improved ICE inhibitors. And, the need to perform relatively random screening of putative compounds using enzymatic assays limited the utility of such methods in identifying inhibitors of ICE. For example, in 1993, when my co-workers at Vertex first began a chemical compound screen to identify ICE inhibitors, they screened about 3000 compounds and found only two that had ICE inhibitory activity (i.e., about a 0.07% success rate).

24. It was within this setting that my co-inventors and I made our invention and defined our claimed pharmacophore. Specifically, our co-workers at Vertex successfully co-crystallized ICE bound to a substrate analog and solved the X-ray diffraction patterns produced by those crystals to obtain ICE crystal structure coordinates. Using those crystal coordinates, my co-inventors and I developed a detailed description in three-dimensional (3D) space of the moieties which comprise the active site of ICE. Using this detailed 3D description as a template, we were able to model an "ICE pharmacophore," a complimentary set of moieties with specific molecular characteristics such that a compound having those characteristics would bind to the ICE active site and be an inhibitor of ICE. The methods and programs we used to design the claimed ICE pharmacophore are

diagramed schematically in **Exhibit N** (attached). The resulting 3D ICE pharmacophore is shown in **Exhibit O**.

25. The concept of modeling or designing a 3D pharmacophore was not new to the art at the time of our invention. For example, **Exhibit P*** (attached) shows NMDA antagonists designed using a proposed pharmacophore model for the NMDA receptor. For further descriptions of pharmacophores for drug design, see, e.g., Y.C. Martin, "3D Database Searching in Drug Design," J. Med. Chem., 25, pp. 2145-54 (1992) (attached as **Exhibit Q**; C. Humblet and J.B. Dunbar, Jr., "3D Database Searching and Docking Strategies," Annu. Rep. Medic. Chem., 28, pp. 275-84 (1993) (attached as **Exhibit R**). In the absence of crystal structure information for a target molecule, a pharmacophore model is typically derived from computer predictions based on the 3D structures of compounds that bind with a known specificity and energy to that target. Such modeling is complicated by the ability of each compound to form an ensemble of low energy 3D conformations, only one of which might tightly bind to the target. In addition, if the modeled compounds bind to only a portion of the target binding site, only that portion of

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* Fig. 11 taken from Payne and Glen, "Molecular Recognition Using a Binary Genetic Search Algorithm," J. Mol. Graphics, 11, p. 74 (1994)).

the pharmacophore will be predicted by the computer. Pharmacophore models derived from computer compound modeling must be repetitively tested and revised to improve their predictive values.

26. The great advantage that has arisen from the disclosure of our claimed ICE pharmacophore is that now anyone of skill in the art -- using the computer modeling programs available at the date of our invention or more recent and improved programs -- can rapidly and accurately select specific ICE inhibitors. The ICE pharmacophore of our invention may be used to screen known compound libraries for ICE inhibitors, or alternatively, may be used to design ICE inhibitor compounds de novo (diagramed schematically in **Exhibits S and T**, respectively; attached).

27. In our experience thus far, greater than 90% of the compounds that we identify by performing the series of steps we described in our application using the claimed ICE pharmacophore of our invention are indeed ICE inhibitors in vitro. This represents a tremendous improvement over the extremely low success rate (<0.1%) we achieved in identifying ICE inhibitors by random screening methods (see page 17, paragraph 23). Thus, the vast majority of compounds selected at random from a typical chemical compound library will fall outside of our claimed ICE

pharmacophore and the majority of compounds which fall within the ICE pharmacophore using molecular modeling techniques will have ICE inhibitory activity.

28. In particular, in Example 1 of our application (pp. 105-108), we describe how to design an ICE inhibitor de novo using our claimed pharmacophore model. To demonstrate the accuracy or our pharmacophore model in designing an ICE inhibitor, we have attached **Exhibit U** (attached). **Exhibit U** shows generally the effects of replacing or removing individually several of the moieties recited in our claims to be important for binding to the ICE active site. Specifically, we see 30-fold to more than 4000-fold losses in the potency of an ICE inhibitor that lacks one of the pharmacophore moieties defined by our model.

29. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section

1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

MARK A. MURCKO

Signed this 25 day of March, 1996 at Cambridge, Massachusetts.

I Hereby Certify that this Correspondence is being Deposited with the U.S. Postal Service as First Class Mail in an Envelope Addressed to: ASSISTANT COMMISSIONER FOR PATENTS WASHINGTON D.C. 20231, on March 28 1996

Thomas Quinones
Name of Person Signing

Signature of Person Signing